# Predicting Acute Copper Toxicity to Valve Closure Behavior in the Freshwater Clam *Corbicula fluminea* Supports the Biotic Ligand Model

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**ABSTRACT:** The objective of this paper is to employ biotic ligand model (BLM) to link between acute copper (Cu) toxicity and its effect on valve closure behavior of freshwater clam *Corbicula fluminea* in order to further support for the BLM that potentially offers a rapid and cost-effective method to conduct the acute toxicity tests for freshwater clam exposed to waterborne Cu. Reanalysis of published experimental data of *C. fluminea* closure daily rhythm and dose–response profiles based on the laboratory-acclimated clams showed that a BLM-based Hill model best described the free $Cu^{2+}$-activity-valve closure response relationships. Our proposed Cu-BLM-*Corbicula* model shows that free ionic form of waterborne Cu binds specifically to a biotic ligand (i.e., clam gills) and impairs normal valve closure behavior, indicating that a fixed-level of metal accumulation at a biotic ligand is required to elicit specific biological effects. With derived mechanistic-based Cu-BLM-*Corbicula* model, we show that the site-specific EC50($t$) and valve closure behavior at any integrated time can be well predicted, indicating that our model has the potential to develop a biomonitoring system as a bioassay tool to on-line measure waterborne Cu levels in aquatic systems. Our results confirm that BLM can be improved to analytically and rigorously describe the bioavailable fraction of metal causing toxicity to valve closure behavior in freshwater *C. fluminea*. We suggest that the Cu-BLM-*Corbicula* model can be used to assist in developing technically defensible site-specific water quality criteria and performing ecological risk assessment and to promote more focused and efficient uses of resources in the regulation and control of metals and the protection of the aquatic ecosystems. © 2007 Wiley Periodicals, Inc. Environ Toxicol 22: 295–307, 2007.

**Keywords:** biotic ligand model; clam; *Corbicula fluminea*; copper; acute toxicity; valve closure behavior

## INTRODUCTION

Wu and Shiau (2002) indicated that a freshwater clam *Corbicula fluminea* extract (or referred to as clam essence) contained more ornithine than that in a chicken or beef essence in that ornithine is attractive as an ingredient of dietary supplements due to in part that ornithine could promote the secretion of the growth hormone and build muscle (Bucci et al., 1990; Davenport et al., 1990; Uchisawa et al., 2004). This evidence makes brackish-water and freshwater *C. fluminea* commercially important and has a high market value to Taiwan's aquaculture (http://www.fa.gov.tw). The

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major *C. fluminea* farming sites are clustered at the Taoyuan, Chunghua, Yunlin, and Chaiyi, located at the western, and Hualien at the eastern coastal areas of Taiwan region, respectively. The major farming strategies of *C. fluminea* are ploy-culture by mixing freshwater and groundwater to reduce freshwater demand. Hung et al. (2001) analyzed the correlations among the trace metal concentrations in bivalves, water, and sediments collected in these areas and indicated that significant correlations were found between bivalves and waterborne metals of copper (Cu), cadmium (Cd), plumbum (Pb), and zinc (Zn). Therefore, if waterborne metals are elevated, pollutant-induced changes in the mobility can occur, which has potentially risks on the health of clam, resulting in reduced market prices and the closure of clam farms.

The biotic ligand model (BLM) is one of a new generation of models that describe the bioavailable fraction of metal causing toxicity in aquatic organisms (Paquin et al., 2002; Niyogi and Wood, 2004). The BLM, which quantifies the affinity and capacity of the gills (biotic ligand, BL) of aquatic organisms to bind metals and relates this binding to acute toxicity, has been proposed as a method for modeling metal toxicity in the aquatic environment based on site-specific water quality parameters. The theory of the BLM evolved from the gill surface interaction model (Pagenkopf, 1983) and the free ion activity model (FIAM) (Campbell, 1995). Using an equilibrium geochemical modeling framework, the BLM incorporates the competition of the free metal ion with other naturally occurring cations (e.g., $Ca^{2+}$, $Na^{+}$, $Mg^{2+}$, $H^{+}$), together with complexation by abiotic ligands (e.g., DOM, chloride, carbonates, sulfide) for binding with the biotic ligand, the site of toxic action on the organisms and the concentration of this metal–BL complex determines the magnitude of the toxic effect, independent of the physiochemical characteristics of the medium. The gill of freshwater organisms bear negatively charged ligands to which cationic metals can bind and constitute the primary sites for toxicity of most metals. Acute Cu toxicity, for example, is associated with the inhibition of sites involved in active $Na^{+}$ uptake at the gills, resulting in death from failure of NaCl homeostasis (Paquin et al., 2002). Water chemistry and associated Cu speciation can greatly affect Cu toxicity. Naturally occurring cations (e.g., $Na^{+}$) can offer protection by competing with $Cu^{2+}$ for binding sites on the gill, whereas naturally occurring anions can bind $Cu^{2+}$, rendering it poorly available to gill sites.

Brown and Markich (2000) and Markich et al. (2003) have employed the extended FIAM to develop a conceptual model to quantify the effect of toxicity of Cd and Cu on valve movement behavior of freshwater bivalve *Hyridella depressa*. Markich et al. (2003) indicated that the valve movement behavior of *H. depressa* exposed to total Cd was directly proportional to the activity of the free metal ion ($Cd^{2+}$) in the linear region of the concentration-response profiles, whereas a weighted function of the activities of the free metal ion and the 1:1 metal hydroxide species (i.e., $2.02 \times Cu^{2+} + CuOH^{+}$) was found for *H. depressa* exposed to total Cu. Markich et al. (2003) therefore claimed that the extended FIAM explained 98% of the variability in valve movement behavior, indicating that the predictive acute toxicity to *H. depressa* supports for the extended FIAM.

In the present research, we focus on the relationship between physiological and behavioral changes of freshwater clam and exposed waterborne Cu levels because the Cu-BLM is at the most advanced stage of development among all developed metal-BLMs (Niyogi and Wood, 2004) and the evidence of the adverse effects of metal toxicity on *C. fluminea* have been presented in numerous studies (Doherty and Cherry, 1988; Markich et al., 2000; Borcherding and Wolf, 2001; Heinonen et al., 2001; Kadar et al., 2001; Markich, 2003; Tran et al., 2003, 2004; Legeay et al., 2005; Qui et al., 2005). The acute BLM for Cu developed in freshwater fish (fathead minnow *Pimephales promeals*) and subsequently adopted for waterflea *Daphnia magna* have been well established respectively by Di Toro et al. (2001), Santore et al. (2001), De Schamphelaere and Janssen (2002), and De Schamphelaere et al. (2002). USEPA in 2000 has already been approved the acute Cu-BLM.

Of those developments of Cu-BLM all showed reasonable success in predicting Cu toxicities (24-h LC50, 120-h LC50, and 48-h EC50) in all test media within a factor of less than 2, and about larger than 90% of the predictions were within a factor of 1.3 from observed values. Furthermore, the acute BLMs for Ag (Paquine et al., 1999; McGeer et al., 2000; Zhou et al., 2005) and acute/chronic BLMs for Zn (Heijerick et al., 2002; Santore et al., 2002; De Schamphelaere and Janssen, 2004; Heijerick et al., 2005) have been published recently. However, only a few fish species (mainly rainbow trout *Oncorhynchus mykiss*) and fathead minnow *Pimephales promelas* have been given emphasis so far for the development of BLM. There is a need to incorporate more species from different fish/shellfish families, especially those present in metal-impacted environment to widen the potential for its application.

The objective of this paper is to employ BLM to link between acute Cu toxicity and its effect on daily valve opening/closure rhythm of *C. fluminea* in order to further support for the BLM that potentially offers a rapid and cost-effective method to conduct the acute toxicity tests for freshwater clam exposed to waterborne Cu. We hope that the proposed Cu-BLM-*Corbicula* system can assist in developing technically defensible site-specific criteria and performing ecological risk assessment and promote more focused and efficient uses of resources in the regulation and control of metals and the protection of the aquatic ecosystems. Thanks to the excellent recordings of typical daily valve opening/closing activities of *C. fluminea* from the previous researchers (Doherty et al., 1987; Ortmann and Grieshaber, 2003; Tran et al., 2003), distributions of the

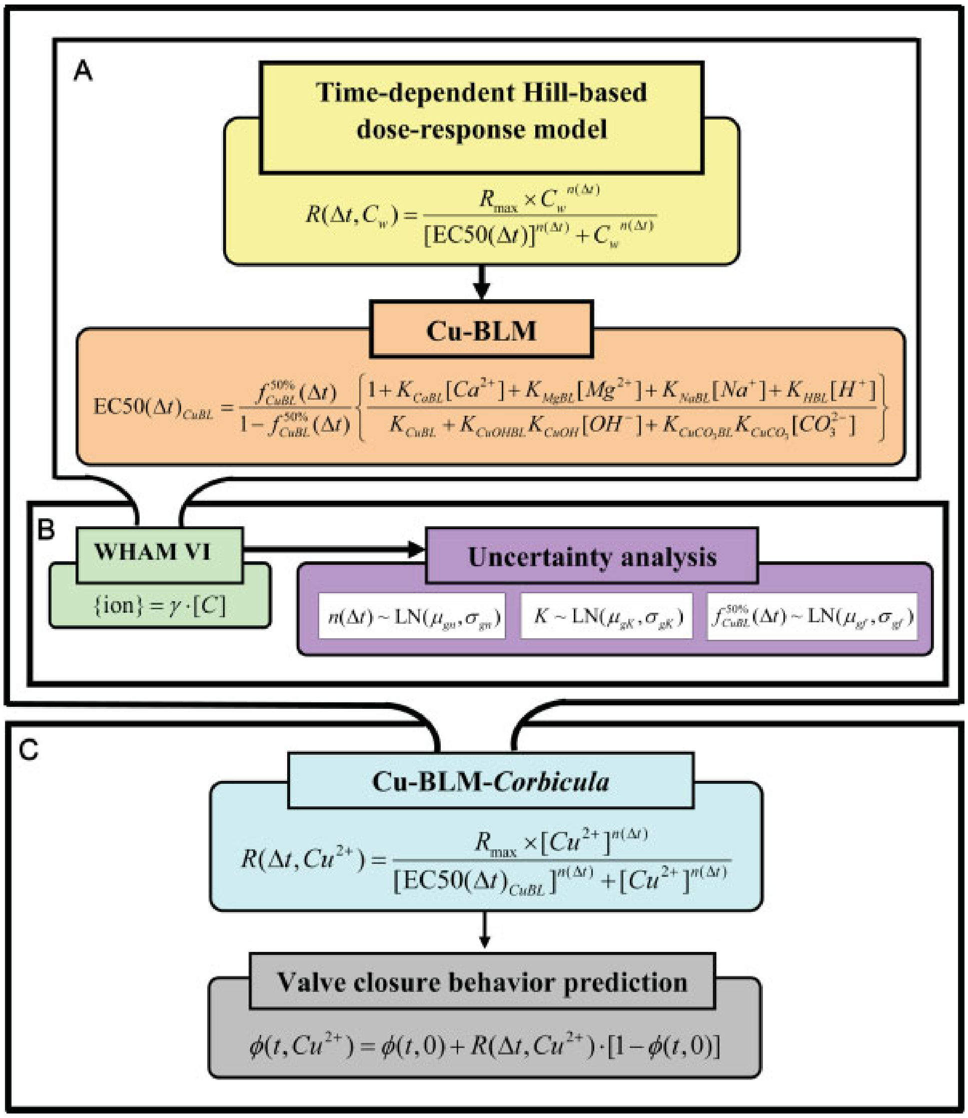


**Fig. 1.** A conceptual algorithm showing an analytical method to derive the Cu-BLM-*Corbicula* model to predict the valve closure behavior in freshwater clam *Corbicula fluminea* in response to waterborne copper (Cu). (See text for detailed descriptions of symbol.) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

daily valve opening/closing rhythm in *C. fluminea* have a well-established sequence framework. The dose–response profiles of the percentages of valve response of *C. fluminea* as a function of Cu at different integrated times and EC50($t$) data have also well presented by Tran et al. (2004).

## MATERIALS AND METHODS

Our approach for developing a BLM-based concentration–time–response model (i.e., Cu-BLM-*Corbicula* model) to predict valve closure behavior in freshwater clam *C. fluminea* is illustrated in Figure 1 and is described in the subsequent sections.

### Cu-BLM-*Corbicula* Model

Brown and Markich (2000) indicated that the extended FIAM is capable of modeling concentration-response experiments from a wider range of water chemistry conditions and potentially offers a more useful tool for evaluating metal–organism interactions. Markish et al. (2000, 2003) used a Hill-based model to account for the

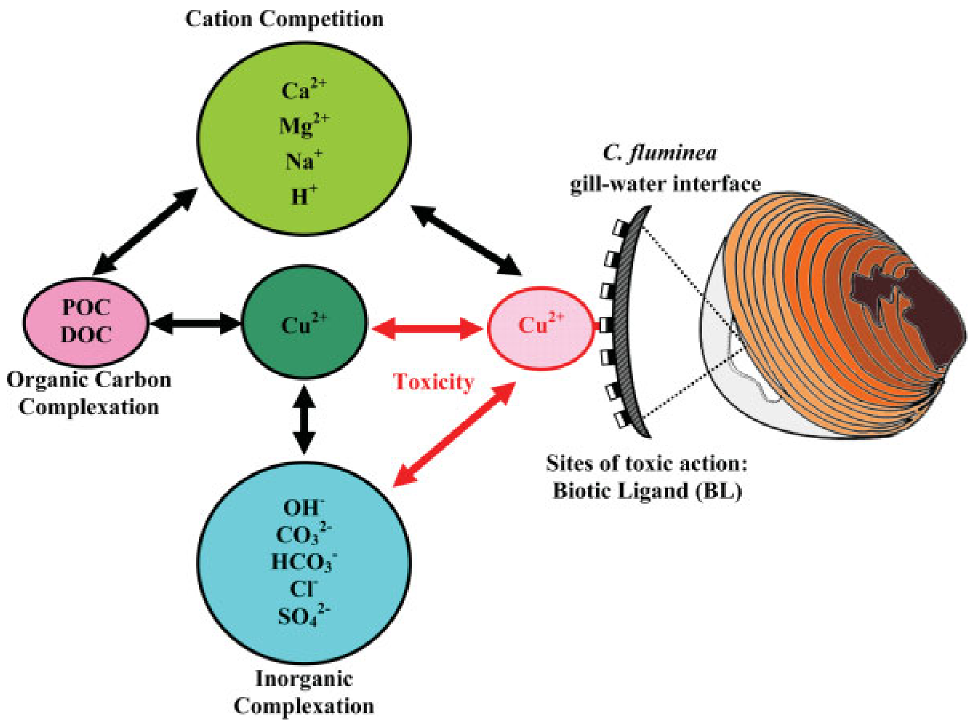


**Fig. 2.** Schematic diagram of acute copper toxicity biotic ligand model (Cu-BLM) for computing the time-dependent 50% effect concentration of copper toxicity to valve closure response of *C. fluminea*. POC, particular organic carbon; DOC, dissolved organic carbon. [Modified from Janssen et al. (2003) and Paquin et al. (2002)]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

relationships between free ion activities of Cu or Cd and biological response of valve closure behavior in freshwater bivalve *H. depressa*. Liao et al. (2005) employed a Hill model-based dose–response function at any given integrated time to describe the valve closure behavior in freshwater clam *C. fluminea* in response to waterborne Cu and Cd,

$$R(\Delta t, C_{\mathrm{w}}) = \frac{R_{\max} \times C_{\mathrm{w}}^{n(\Delta t)}}{[\mathrm{EC50}(\Delta t)]^{n(\Delta t)} + C_{\mathrm{w}}^{n(\Delta t)}}, \qquad (1)$$

where $R(\Delta t,\ C_{\mathrm{w}})$ is the measured response (% response) at any give $\Delta t$, EC50($\Delta t$) is the time-dependent metal concentration yielding half of maximal response of $R_{\max}$ ($\mu$g L$^{-1}$), $C_{\mathrm{w}}$ is the metal concentration in water ($\mu$g L$^{-1}$), and the exponent $n(\Delta t)$ is a time-dependent Hill coefficient, which is a measure of cooperativity. A value of $n > 1$ indicates positive cooperativity.

The Cu-BLM framework applied to *C. fluminea* is diagrammed in Figure 2. Free $Cu^{2+}$ bind to the BL of *C. fluminea* gill, which may be transport sites and/or toxic action sites. The concentration of $Cu^{2+}$ bound to the BL is directly proportional to the toxic effect and is independent of the physiochemical characteristics of the medium. The $Cu^{2+}$-activity is reduced by binding to organic (e.g., particulate organic carbon (POC) and dissolved organic carbon (DOC)) and inorganic ligands that reduce the bioavaliability and thus reduce the toxicity. Inorganic ligands include $OH^-$ and $CO_3^{2-}$. The concentrations of these ligands are increased at increasing pH and alkalinity of the medium, respectively. Cations in water can compete with $Cu^{2+}$ for the BL, which also reduces bioavailability to the BL and thus reduces the toxicity. In light of the concept of BLM (Fig. 2), a modified version of the basic Hill model equation can be developed, which allows free $Cu^{2+}$-activity and BLM-based EC50 model incorporate into Eq. 1 and is referred to as the Cu-BLM-*Corbicula* model,

$$R(\Delta t, \mathrm{Cu}^{2+}) = \frac{R_{\max} \times [\mathrm{Cu}^{2+}]^{n(\Delta t)}}{[\mathrm{EC50}(\Delta t)_{\mathrm{CuBL}}]^{n(\Delta t)} + [\mathrm{Cu}^{2+}]^{n(\Delta t)}}, \qquad (2)$$

where $R(\Delta t,\ Cu^{2+})$ is the time-dependent valve response based on $Cu^{2+}$-activity [$Cu^{2+}$] (M) and EC50$(\Delta t)_{\mathrm{CuBL}}$ is the time-dependent BLM-predicted acute Cu EC50 value (M).

On the basis of the refined Cu-BLM scheme (De Schamphelaere et al., 2002), EC50$(\Delta t)_{\mathrm{CuBL}}$ in Eq. 2, taking into account the bioavailability and toxicities of $CuOH^+$ and $CuCO_3$, has the form as

$$EC50(\Delta t)_{\mathrm{CuBL}} = \frac{f_{\mathrm{CuBL}}^{50\%}(\Delta t)\{1 + K_{\mathrm{CaBL}}[\mathrm{Ca}^{2+}] + K_{\mathrm{MgBL}}[\mathrm{Mg}^{2+}] + K_{\mathrm{NaBL}}[\mathrm{Na}^{+}] + K_{\mathrm{HBL}}[\mathrm{H}^{+}]\}}{\left(1 - f_{\mathrm{CuBL}}^{50\%}(\Delta t)\right)\{K_{\mathrm{CuBL}} + K_{\mathrm{CuOHBL}}K_{\mathrm{CuOH}}[\mathrm{OH}^{-}] + K_{\mathrm{CuCO_3BL}}K_{\mathrm{CuCO_3}}[\mathrm{CO_3^{2-}}]\}}, \tag{3}$$

where $f_{\mathrm{CuBL}}^{50\%}(\Delta t)$ is the time-dependent fraction of the total number of Cu binding sites occupied by Cu at 50% effect; $K_{\mathrm{CuBL}}$, $K_{\mathrm{CaBL}}$, $K_{\mathrm{MgBL}}$, $K_{\mathrm{NaBL}}$, $K_{\mathrm{HBL}}$, $K_{\mathrm{CuOHBL}}$, $K_{\mathrm{CuCO_3BL}}$ are the stability constants for the binding of these cations to the BL ($M^{-1}$); $K_{\mathrm{CuOH}}$ and $K_{\mathrm{CuCO_3}}$ are the stability constants for the formations of the $CuOH^{+}$ and $CuCO_3$, respectively ($M^{-1}$); and [ion] denotes the activity of each ion of water chemistry characteristics (M).

The evaluation of Eq. 3 for predicting time-dependent EC50 values expressed as $[Cu^{2+}]$ requires values of cation activities and known stability constants associated the calculated fraction of the BL sites occupied by Cu. Practically, Eq. 3 is integrated into a Windemere Humic Aqueous Model (WHAM) (Tipping, 1994) by adding stability constants for the binding of metal species (i.e., $Cu^{2+}$, $CuOH^{+}$, and $CuCO_3$) and competing cations ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, and $H^{+}$) onto the BL. Through this linkage of Eqs. 2 and 3 and WHAM associated with calculated $f_{\mathrm{CuBL}}^{50\%}(\Delta t)$ value that can be estimated by fitting Eq. 3 to the published EC50($\Delta t$) data, we could predict a site-specific concentration–time–response profile of valve closure behavior of *C. fluminea* in response to waterborne Cu. The published dose–response profiles and EC50($\Delta t$) data obtained from Tran et al. (2004) in that the bioassay were carried out in a flow-through system by using 60 *C. fluminea* (averaged fresh weight without the shell was 0.47 ± 0.01 g) with groundwater at 15 ± 0.5 °C and pH = 8.2 ± 0.1 and its ionic composition was 0.470 meq $L^{-1}$ $Ca^{2+}$, 0.327 meq $L^{-1}$ $Mg^{2+}$, 1.350 meq $L^{-1}$ $Na^{+}$, 0.092 meq $L^{-1}$ $K^{+}$, 0.001 meq $L^{-1}$ $NH_4^{+}$, 1.910 meq $L^{-1}$ $HCO_3^{-}$ + $CO_3^{2-}$, 1.030 meq $L^{-1}$ $Cl^{-}$, 0.002 meq $L^{-1}$ $NO_3^{-}$, and 0.073 meq $L^{-1}$ $SO_4^{2-}$.

## Valve Closure Behavior Predictions

We could link the Cu-BLM-*Corbicula* model shown in Eq. 2 and the fitted model of daily rhythm valve opening/closing to predict the bivalve closure behavior in response to waterborne Cu,

$$\phi(t, \mathrm{Cu}^{2+}) = \phi(t,0) + (1-\phi(t,0))R(\Delta t, \mathrm{Cu}^{2+}), \tag{4}$$

where $\phi(t, Cu^{2+})$ is the daily rhythm function of valve closure at time $t$ in response to $Cu^{2+}$-activity, $\phi(t, 0)$ is the daily rhythm function of valve closure exposed to unpolluted water, and $R(\Delta t, Cu^{2+})$ is the Cu-BLM-*Corbicula* model shown in Eq. 2. The daily rhythm function of valve closure exposed to unpolluted water $\phi(t, 0)$ has the form modeled as a three-parameter lognormal model (Liao et al., 2005),

$$\phi(t,0) = \begin{cases} \phi_1(t,0) = 12.3\exp\left[-0.5(\ln(\mathrm{t}/4)/0.20)^2\right] + 3.8, 0 \le t \le 7, r^2 = 0.84, \\ \phi_2(t,0) = 14.8\exp\left[-0.5(\ln(t/18.2)/0.083)^2\right] + 3.6, 7 \le t \le 24, r^2 = 0.92. \end{cases} \tag{5}$$

This model approximation has the form of a bimodal distribution of the daily rhythm of valve closure that is separated at 07:00 a.m. based on the suggestion by Tran et al. (2003). Tran et al. (2003) used 71 *C. fluminea* (averaged fresh weight without the shell was 0.76 ± 0.03 g) over a period of 50 days in a flow-through system at water temperature of 15 ± 0.5 °C with pH ranged from 7.8 to 8.0 and fed continuously with a unicellular algae *Scenedesmus subspicatus* to determine the daily valve opening/closing rhythm.

## Uncertainty Analysis

### Model Parameterization

Parameterization of the Cu-BLM-*Corbicula* model involved selecting datasets and deriving input distributions. Data were sorted by reported statistical measures, e.g., mean, standard deviation, standard error, etc. We used the chi-square ($\chi^2$) and the Kolmogorov-Smirnov (K-S) statistics to optimize the goodness-of-fit of distributions. We performed Statistica®

**TABLE I. Measured pH and temperature values with the ion activities of key water chemistry characteristics calculated by WHAM VI from published measured ion free ion concentrations for three selected clam farm locations**

| | | | Ion Activities (mM) | | | | |
|---|---|---|---|---|---|---|---|
| Clam Farm Location | pH | Temp. (°C) | $Ca^{2+}$ | $Mg^{2+}$ | $Na^{+}$ | $Cl^{-}$ | $SO_4^{2-}$ |
| Taoyuan | 7.19 ± 0.36[a] | 24.9 ± 1.0 | 0.62 ± 0.24 | 0.21 ± 0.19 | 0.13 ± 0.076 | 0.45 ± 0.23 | 0.27 ± 0.19 |
| Changhua | 8.01 ± 0.19 | 29.3 ± 0.9 | 0.41 ± 0.14 | 0.34 ± 0.075 | 0.43 ± 0.23 | 0.40 ± 0.25 | 0.098 ± 0.15 |
| Hualien | 7.80 | 30.5 | 0.36 | 1.17 | 12.28 | 55.57 | 1.42 |

[a] Mean ± SD ($n = 3$).

software package (StatSoft, Tulsa, OK) to analyze data and to estimate distribution parameters.

## Measured Metal Ion Concentration

Distributions of the published measured metal ion concentrations were fitted by the polled field observations obtained from selected clam farms located at Taoyuan, Changhua, and Hualien of Taiwan region (Table I). Metal analyses were carried out by atomic absorption spectrophotometry using a Perkins Elmer model 5000 atomic absorption spectrophotometer (Perkins-Elmer, Shelton, CT) equipped with a graphite furnace. We determined that the lognormal distribution model fits the observed data of ion activity concentrations in three selected clam farms favorably. All variables modeled as the lognormal distributions from which geometric mean and geometric standard deviation for each variable was calculated (Fig. 3). We performed WHAM Version 6 (WHAM VI, Centre for Ecology and Hydrology, Lancaster, UK) to calculate the activities of the competing cations considered in this study.

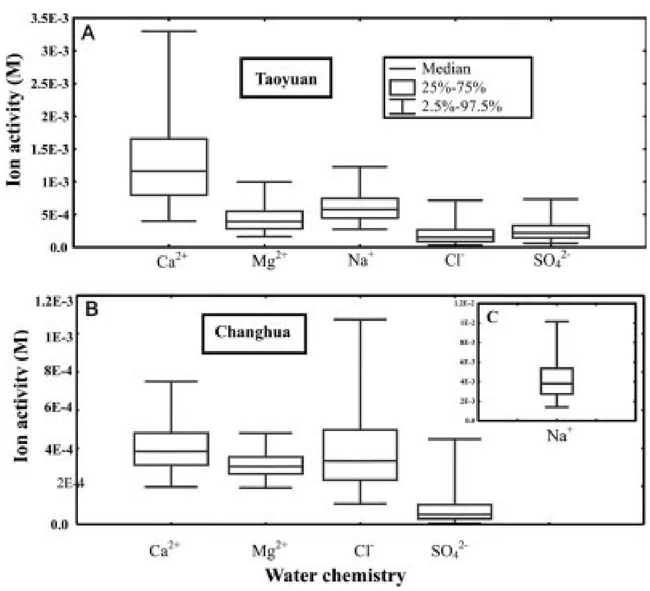


**Fig. 3.** Box and whisker plot representations of ion activities of key water chemistry characteristics of groundwater measurements from selected clam farm locations of (A) Taoyuan and (B) Changhua.

## Parameters in Cu-BLM-*Corbicula* Model

At present, there are three available versions of the acute Cu-BLM that are developed for fathead minnow *P. promelas* and *D. magna*, respectively. Niyogi and Wood (2003) summarized the estimated stability (or affinity) constants (log *K*) of BL–cation and inorganic complexes used in different versions of the Cu-BLM. To account for the uncertainty/variability of log *K* in different versions, we determined a lognormal distributions for log *K*s based on K-S statistics (Table II). In applying dose–response relationships derived from experimental studies adopted from Tran et al. (2004), we must consider the limitations of the data and account for the inherent uncertainty that arises from a number of sources, including the limited number of observations and limited sample size within treatment sets. To account for this uncertainty, we constructed distributions for the input variables of $f^{50\%}_{CuBL}(\Delta t)$ and $n(\Delta t)$ values by fitting Cu-BLM-*Corbicula* model to published dose-percentage valve response curves and EC50($\Delta t$) data from Tran et al. (2004). We also determined a lognormal distribution for $f^{50\%}_{CuBL}(\Delta t)$ and $n(\Delta t)$ values based on K-S statistics, and incorporated the distributions into the Monte Carlo simulation to obtain 2.5th- and 97.5th-percentiles as the 95% confidence interval (CI). We used TableCurve 2D (Version 5, AISN Software, Mapleton, OR) to optimal fit the estimated distribution data of $f^{50\%}_{CuBL}(\Delta t)$ and $n(\Delta t)$ to obtain the optimal statistical models.

**TABLE II. Distributions and point values of affinity constants (log *K*, $M^{-1}$) of biotic ligand-cation and inorganic complexes used in the present Cu-BLM-*Corbicula* model**

| log *K* | Point Value[a] | log *K* | Distribution[b] |
|---|---|---|---|
| log $K_{MgBL}$ | 3.58 | log $K_{CaBL}$ | LN(3.53, 1.03) |
| log $K_{HBL}$ | 5.40 | log $K_{NaBL}$ | LN(3.09, 1.04) |
| log $K_{CuOHBL}$ | 7.45 | log $K_{CuBL}$ | LN(7.70, 1.06) |
| log $K_{CuOH}$ | 6.48 | log $K_{CuCO_3}$ | LN(6.66, 1.02) |
| log $K_{CuCO_3BL}$ | 7.01 | | |

[a] Adopted from Niyogi and Wood (2004).

[b] Lognormal distribution with a geometric mean and a geometric standard deviation.

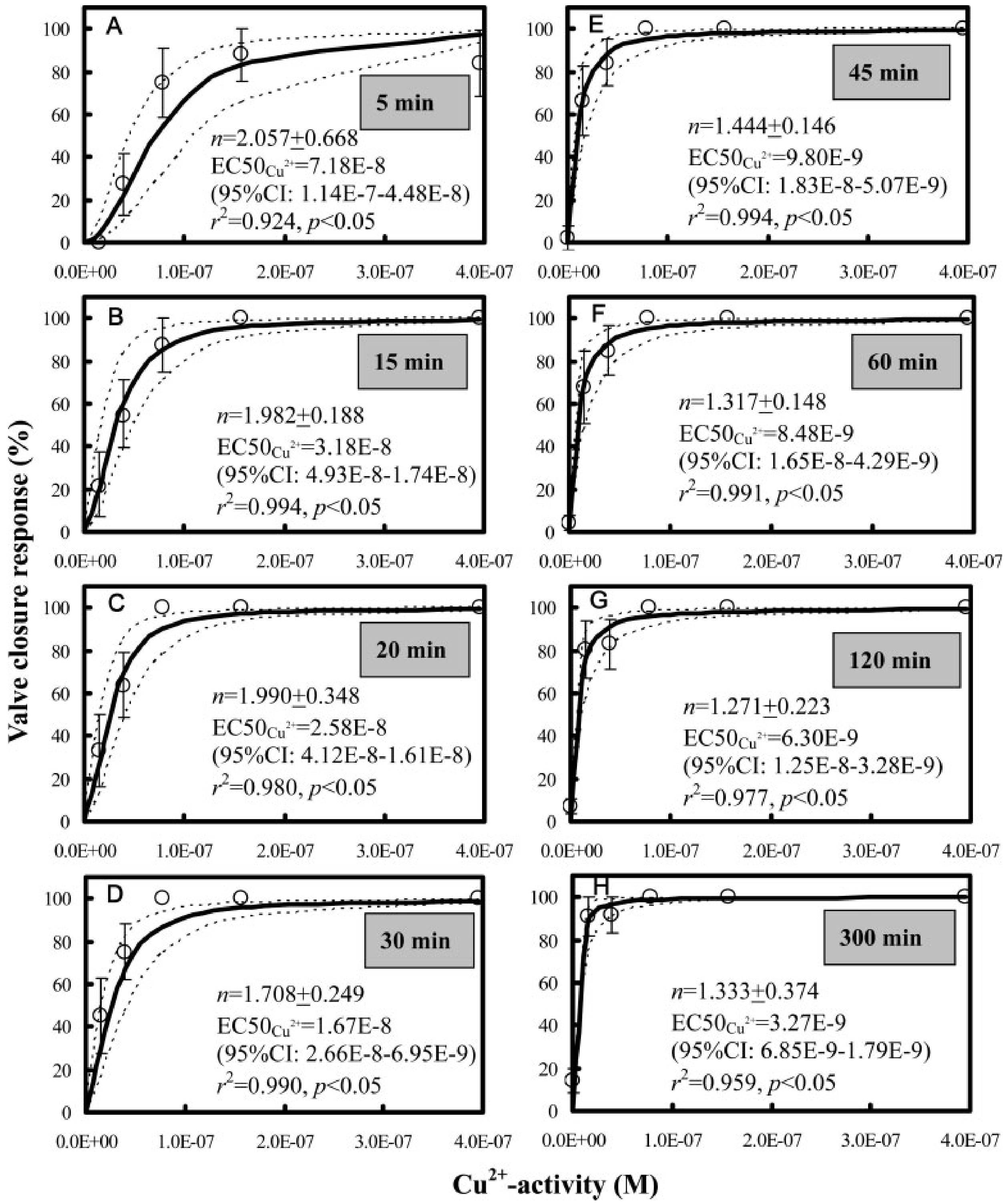


**Fig. 4.** Reconstructed concentration–response profiles with 95% confidence interval for the percentage of valve closure response as a function of $Cu^{2+}$ activity characterized by a nonlinear three-parameter Hill equation model at different integrated times of 5, 15, 20, 30, 45, 60, 120, and 300 min. The measurements are shown with open circle with error bars denoting one standard deviation from the mean.

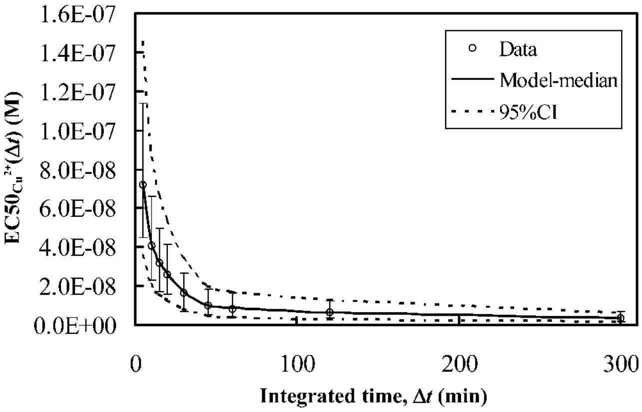


**Fig. 5.** Comparison between measured and Cu-BLM-based predicted EC50(*t*) values. Error bars represent one standard deviation from the mean.

## Monte Carlo Analysis

To quantify this uncertainty and its impact on the predictive capacity of Cu-BLM-*Corbicula* model, we implemented a Monte Carlo simulation that includes input distributions for the parameters of the derived dose–time–response function. To test the convergence and the stability of the numerical output, we performed independent runs at 1, 4, 5, and 10 thousand iterations, with each parameter sampled independently from the appropriate distribution at the start of each replicate. Largely because of limitations in the data used to derive model parameters, inputs were assumed to be independently. The result shows that 10,000 iterations are sufficient to ensure the stability of results. We employed Crystal Ball® software (Version 2000.2, Decisioneering, Denver, CO) to implement the Monte Carlo simulation.

# RESULTS

## Cu-BLM-*Corbicula*-Based Dose–Response Relationships

The time-dependent Hill-based dose–response model and a 10,000 Monte Carlo simulation provided an adequate fit for the published data at different Cu concentration (0, 20, 50, 100, 200, and 500 $\mu$g $L^{-1}$) and different integrated time (5, 15, 20, 30, 45, 60, 120, and 300 min) ($\chi^2$ goodness-of-fit, $P > 0.5$) (Fig. 4). The $n(\Delta t)$ and $EC50_{Cu^{2+}}(\Delta t)$ values clearly show that there were profound differences in sensitivity to Cu in different integration times of response. Regression lines from the nonlinear Hill three-parameter model transformations of percent valve closure response *versus* water $Cu^{2+}$-activity curves had good fit as judged by high $r^2$ values (0.924–0.994, $P < 0.05$). The time-dependent Hill coefficient $n(\Delta t)$ for Cu concentration to valve response (1.271–2.057) was indicative of positive cooperativity. Based on our fitted $Cu^{2+}$-activity–time–response model (Fig. 4), the estimated $EC50_{Cu^{2+}}(\Delta t)$values were $1.67 \times 10^{-8}$, $8.48 \times 10^{-9}$, and $6.30 \times 10^{-9}$ M for valve response times of 30, 60, and 120 min. Therefore, low concentrations of Cu caused a significant change in the valve position, suggesting that valve position is suitable for a biologically sensitive endpoint.

The time-dependent fraction of the total number of Cu binding sites occupied by Cu at 50% effect, $f^{50\%}_{\text{CuBL}}(\Delta t)$, could be estimated by fitting Cu-BLM (Eq. 3) to published EC50($\Delta t$) data from Tran et al. (2004) associated with known ionic compositions and log $K$ values shown in Table II (Fig. 5). Figure 6(A) gives the relationship between estimated $f^{50\%}_{\text{CuBL}}(\Delta t)$ and response integrated time ($\Delta t$) in that fitted $f^{50\%}_{\text{CuBL}}(\Delta t)$ has a form as

$$f^{50\%}_{\text{CuBL}}(\Delta t) = 0.187 + 0.693 \exp(-\Delta t/a), \tag{6}$$

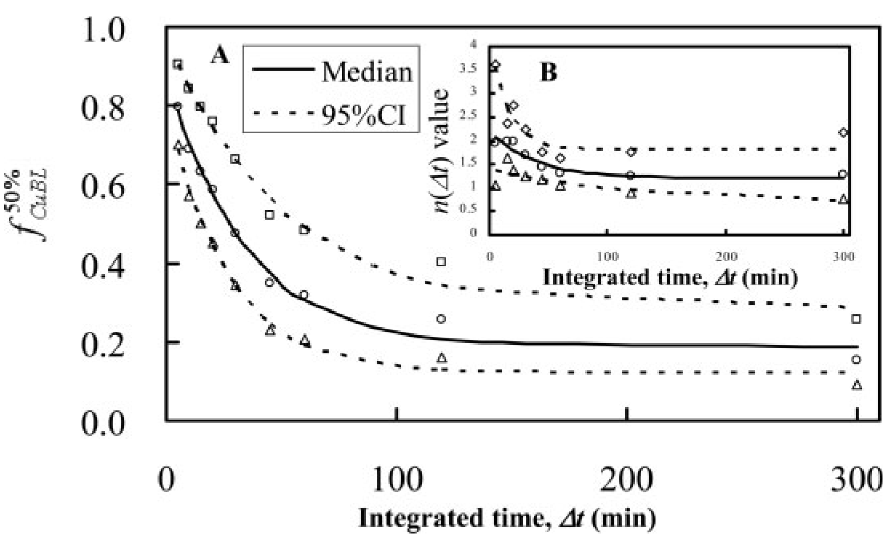


**Fig. 6.** Relationship between predicted (A) time-dependent fraction of the total number of Cu binding sites occupied by Cu at 50% effect $f^{50\%}_{\text{CuBL}}(\Delta t)$ and (B) time-dependent Hill coefficient $n(\Delta t)$ and integrated time ($\Delta t$).

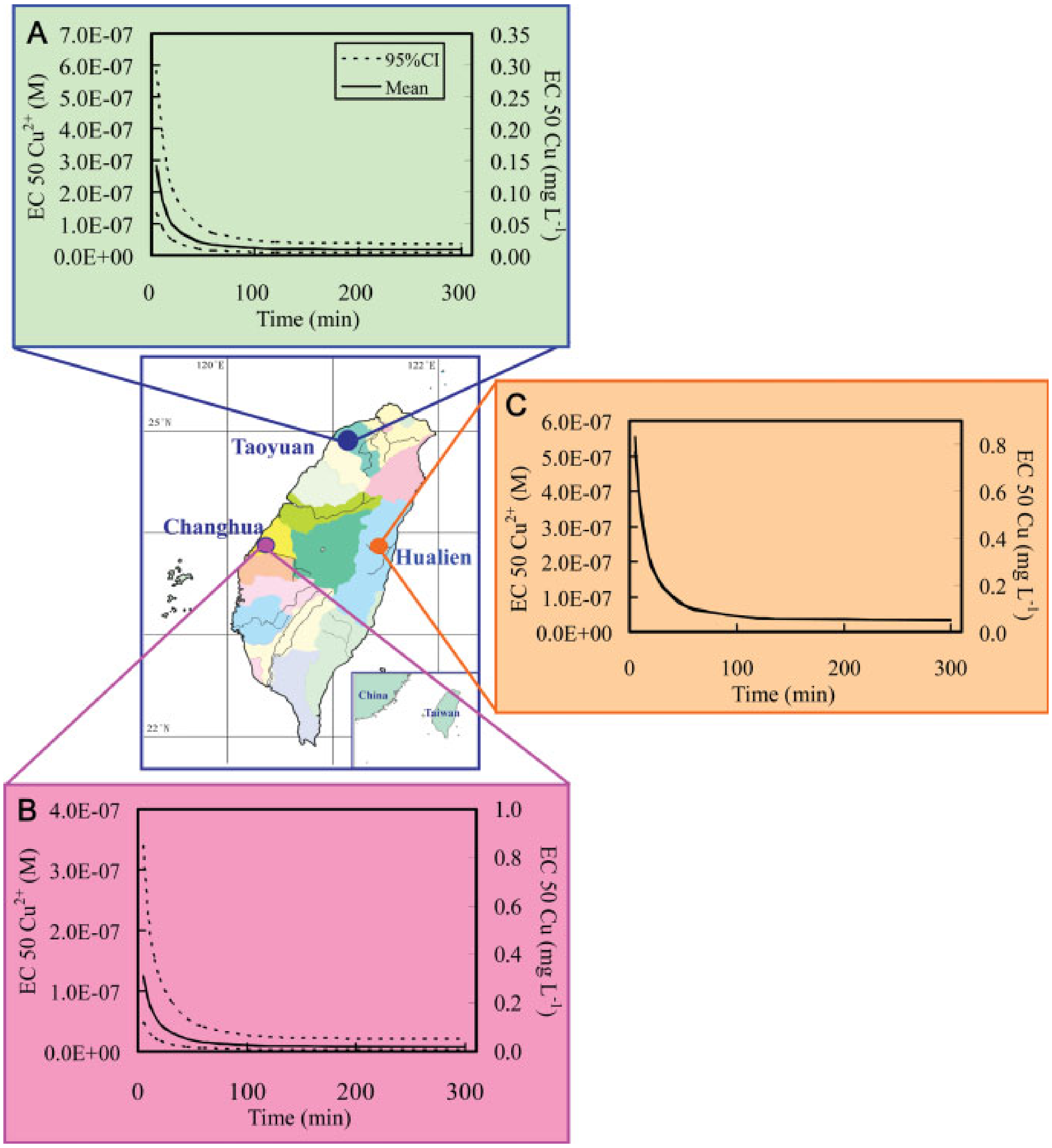


**Fig. 7.** Predicted site-specific EC50(*t*) values for three selected clam farms of (A) Taoyuan, (B) Changhua, and (C) Hualien for *C. fluminea* in response to different water chemistry characteristics of groundwater based on Cu-BLM-*Corbicula* model. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

with $a = 34.298$ (95% CI: 25.58–48.31) ($r^2 = 0.998$). The relationship between estimated $n(\Delta t)$ (Fig. 4) and response integrated time ($\Delta t$) also shown in Figure 6(B) and has a form as $n(\Delta t) = 1.221 + 0.988\exp(-\Delta t/b)$ with $b = 37.703$ (95% CI: 17.29–71.75) ($r^2 = 0.891$). Figure 5 also shows a comparison between measured and predicted $EC50_{Cu^{2+}}(\Delta t)$, indicating measured data all fall within the 95% CI range.

## Predicted Site-Specific EC50(*t*) and Valve Closure Behavior

We perform Eq. 3 with $f^{50\%}_{CuBL}(\Delta t) - \Delta t$ relationship shown in Eq. 6 to predict site-specific EC50($\Delta t$) for *C. fluminea* in response to waterborne Cu for selected clam farms located at Taoyuan, Changhua, and Hualien (Fig. 7). Figure 7 indicates that predicted 30 min-EC50s are 0.069 (95% CI:

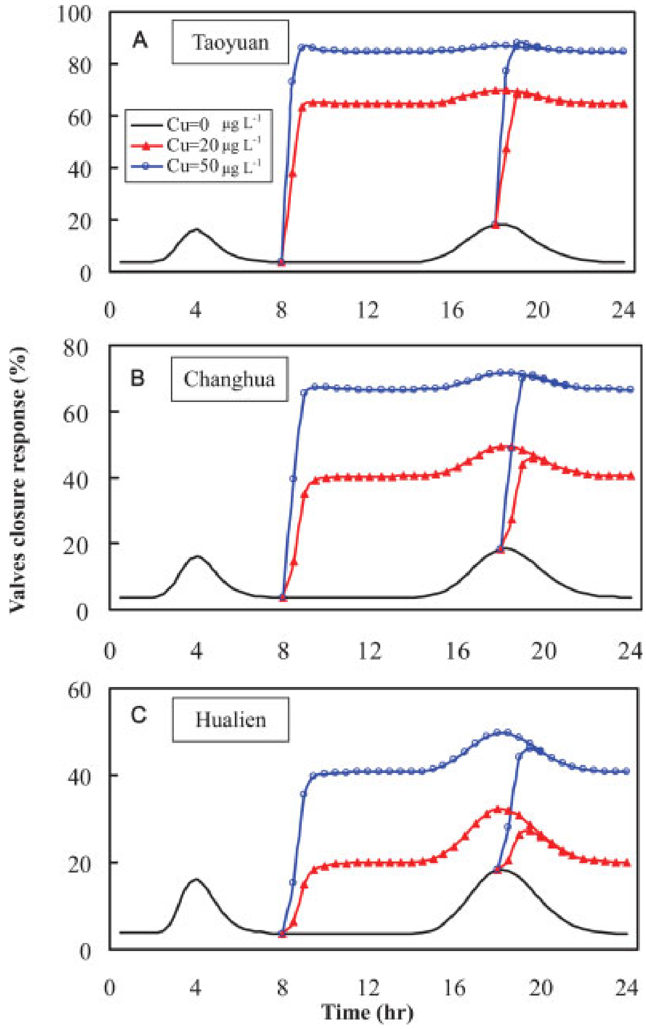


**Fig. 8.** Simulations of valve closure behavior of freshwater *C. fluminea* in response to different waterborne copper concentrations of 20 and 50 $\mu$g $L^{-1}$ at two exposure time periods of 08:00 and 18:00 h for three selected clam farms of (A) Taoyuan, (B) Changhua, and (C) Hualien. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

0.033–0.144), 0.032 (95% CI: 0.012–0.082), and 0.137 $\mu$M, whereas 120 min-EC50s are 0.02 (95% CI: 0.01–0.042), 0.009 (95% CI: 0.003–0.024), and 0.04 for Taoyuan, Changhua, and Hualien clam farms, respectively, indicating that valve closure behavior of *C. fluminea* in Changhua clam farm is more sensitive than that in Taoyuan and Hualien.

Figure 8 demonstrates the simulations of daily rhythm of valve closure subjected to site-specific waterborne Cu (20 and 50 $\mu$g $L^{-1}$) concentrations at different exposure time periods for three selected clam farms, indicating that the proposed Cu-BLM-*Corbicula* model can quantitatively describe the site-specific valve closure behavior when clam exposed to waterborne Cu. Figure 8 also indicates that Taoyuan clam farm has the largest percentage of valve closure of clam comparing with that in Changhua and Hualien. We thus suggest that Cu-BLM-*Corbicula* model can be used to test the bivalve biological response ability to close its shell as an alarm signal to reflect clam's health when exposed to waterborne Cu.

## DISCUSSION

### Alternatives to Cu-BLM-*Corbicula* Model

The direct influence of the environment on valve closure behavior of *C. fluminea* may be particularly pronounced in aquatic ecosystems where the clams are commonly exposed to a complex environmental condition. Alternations in variables such as water chemistry, temperature, and dissolved gases, to name a few, will have profound effects on biological responses. Equally important is the influence that such dramatic variations in environment, such as water chemistry, will have on the biological behavior or chemical speciation of a toxicant. These environmental factors may be considered (in)direct determinants of biological behavior in that they exert their effects independent of the biological entity, by modifying the bioavailability and toxicity of toxicants before interacting with the organisms. Research over the last two decades on metal toxicity in aquatic organisms has elucidated the importance of speciation on toxicity, concluding that in most cases the free ion form of a metal is generally most toxic to aquatic organisms (Campbell, 1995; Borgmann et al., 2005; De Schamphelaere et al., 2005).

BLM is such a model to incorporate the importance of metal speciation as a determinant of toxicity. Our Cu-BLM-*Corbicula* model shows that free ionic form of waterborne Cu binds specifically to a BL (i.e., clam gills) and impairs normal valve closure behavior, indicating that a fixed-level of metal accumulation at a BL is required to elicit specific biological effects. The magnitude of these perturbations is dependent on the extent to which Cu is bioavailable that is largely influenced by water chemistry. BLM therefore has the potential to provide a relatively simple, inexpensive, and scientifically defensible way of predicting metal toxicity. Our work suggests that the biological endpoint such as valve closure behavior can be implemented into the conceptual framework of the BLM and used to predict toxicity in a similar *a priori* fashion. This approach offers the advantage in that the endpoint used in the development of Cu-BLM-*Corbicula* model is based on known interactions of the free metal ion with the actual sites of toxic action.

Ortmann and Grieshaber (2003) pointed out that the freshwater *C. fluminea* acclimated at cold water temperatures during winter changed their valve movements and closure rhythm to depress the metabolic rate for reducing their heat dissipation and oxygen consumption. Tran et al. (2001), Markich et al. (2003), Qiu et al. (2005), and Legeay et al. (2005) indicated that some physical and chemical variables in water such as temperature, pH, DOC, hardness (expressed as $Ca^{2+}$), dissolved oxygen level, which also affected the behavior and activity of the valve opening/closing to a greater or lesser extent. Thus in the present operational and realistic constraining conditions, we have to consider the interactive effects of the dose–response and valve closure behavior of *C. fluminea* exposed to various water chemistry characteristics to accurately implement the Cu-BLM-*Corbicula* model. Seasonal variations have shown the need to set different parameters related to the seasonal conditions (Ortmann and Grieshaber, 2003). The most suitable farming conditions for *C. fluminea* in Taiwan region were under the range values of temperature 15–25 °C, pH 7.0–8.5, salinity 0–2 ppt, and D.O. 3–4 ppm (http://shell.sinica.edu.tw/english/program.php).

We adopted the published experimental data of *C. fluminea* closure daily rhythm and dose–response profiles from Tran et al. (2004) based on the laboratory-acclimated clams to derive Cu-BLM-*Corbicula* model. According to the published data, the experimental conditions of mean water temperature of 15 ± 0.5 °C with pH ranged from 7.8 to 8.0 that were different from the present farming environment of freshwater *C. fluminea* in Taiwan region. The other further limitations must be taken into consideration, i.e., the uncertainty/variability problems provoked by spatial and local conditions, e.g., photoperiod, light intensity and tropic additions, which may also affect the daily rhythm activity (Englandlund and Henio, 1994).

## Implications for Environmental Risk Assessment

Valve movement is a characteristic feature of many bivalves and part of their natural behavior and it cannot be neglected as an important physiological factor for their survival. Changes in the valve movement rhythm of bivalves can therefore be used as a suitable endpoint in ecotoxicological risk assessment. The *C. fluminea* are filter-feeder animals. They extend siphon from their bivalve shells to filtrate waterborne plankton or organic matter for uptake. Siphon extension related to the magnitude of shell gape (%) that was proportioned to the valve position as well as percentage of the shell span (Ortmann and Grieshaber, 2003). When valve closure behavior in response to waterborne contaminants reduced filtrating-uptake activity by closing their shells to escape toxicant damage and exclude themselves from the outside contaminated environment for maintaining their biotic faculty and increasing their survivability (Wildridge et al., 1998; Kadar et al., 2001).

On the basis of the valve position, we may use two different valve responses with respect to behavioral activities of the clam as biological endpoints, i.e., closure and filtration decreasing for BLM development. Using different biological endpoints to develop BLM would be ideal for formulating predictive models of chronic low-level metal exposures in aquatic ecosystems. The BLM offers a conceptual framework for integrating the influence of environmental factors such as water chemistry and bioavailability on biological response as monitored using valve movement behavior. Developing novel methods through BLM for interpreting biological response data will increase its utility in environmental risk assessment of toxicant exposure for aquatic species (Janssen et al., 2003).

Meaningful water quality criteria (WQC) are needed to serve as a basis for development and implementation of a site-specific risk management strategy that will protect the aquatic environment, whereas at the same time result in the cost-effective implementation of control measures. Janssen et al. (2000) pointed out that neither total nor dissolved aqueous metal concentrations are good predictors of metal bioavailibility and toxicity and are inadequate to accurately assess the potential impact of metals on the ecological quality of ecosystems. From the perspective of the aquatic ecosystems, rather than developing a single-value waterborne metal concentration for establishing the WQC, it is better to derive a mechanistic model that explicitly incorporates the factors controlling bioavailability and bioaccumulation to enhance predictive ability to protect aquatic species. Therefore, the BLM integrates knowledge of water chemistry with physiological mechanisms of toxicity to generate a site-specific assessment of the toxicity of a given metal to the biota therein and provides a direct and quantitative method for the evaluation of metal bioavailability in ecological risk assessment as a function of water chemistry and organism sensitivity to overcome frequently over-protective, and occasionally under-protective on site-specific WQC, thereby providing a means for estimating the effect of site-specific factors on metal toxicity.

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